Somatic variant calling and interpretation in the context of cancer

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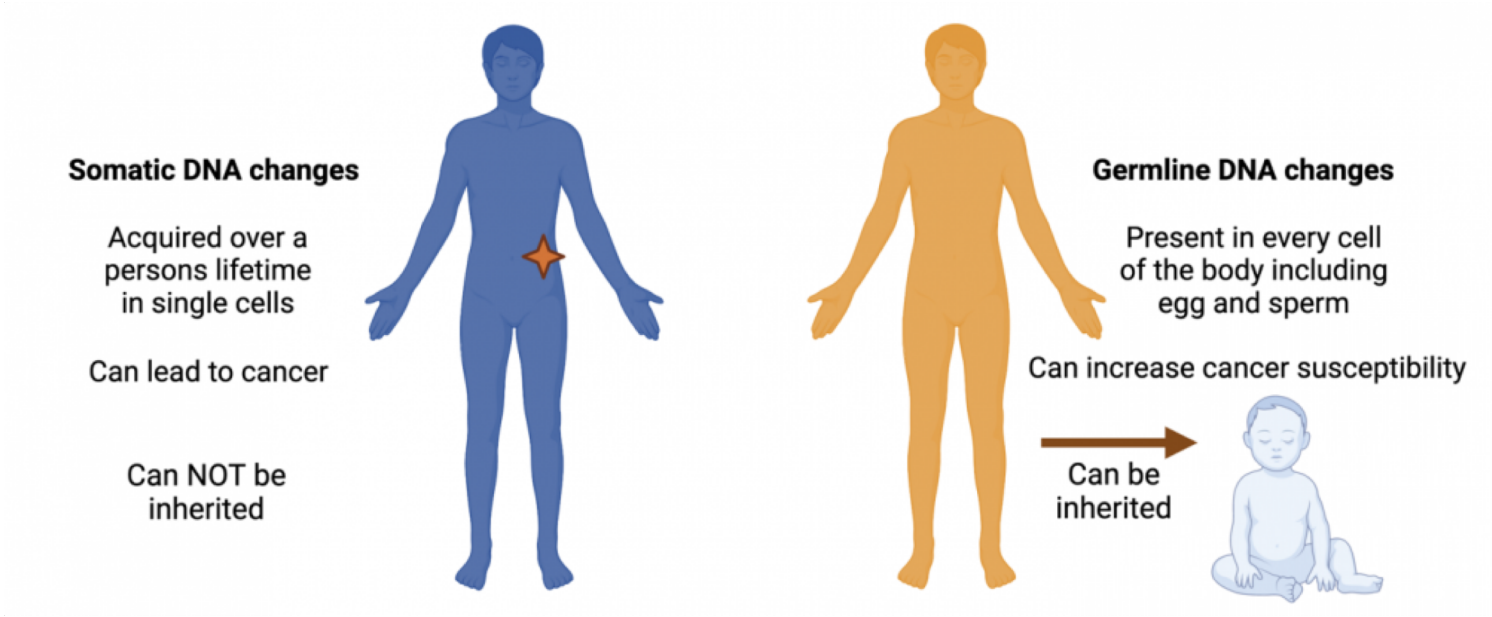
Learning outcomes – part I

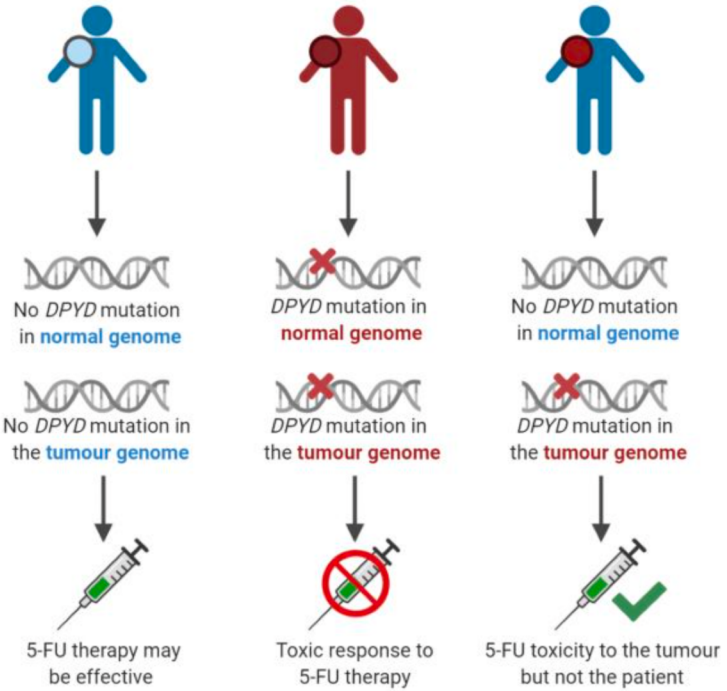
- Somatic mutations - why do we care about them?
- Which properties of tumor samples make somatic mutation calling challenging?
- Somatic variant calling principles – tumor/control vs. tumor-only
- Perspective: short-read sequencing – SNVs/InDels

Germline versus somatic mutations



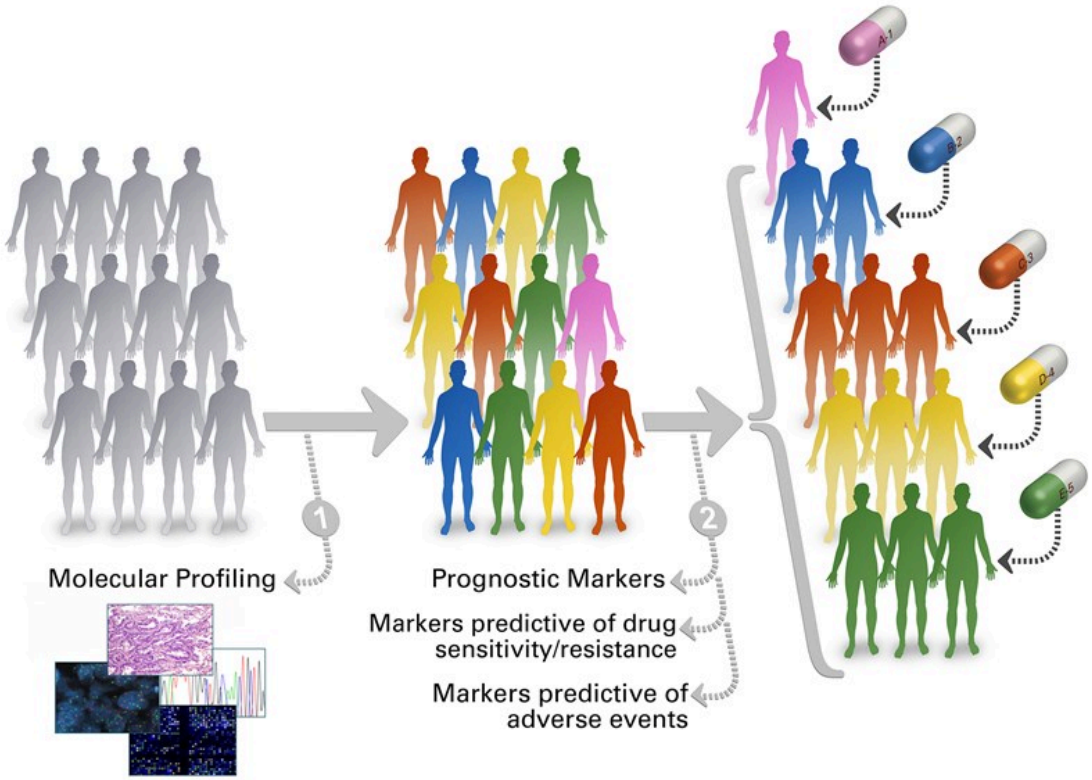
https://www.bcgsc.ca/news/tumor-sequencing-panel-screens-both-somatic-and-germline-changes-clinical-significance

Mutations inform upon treatment options

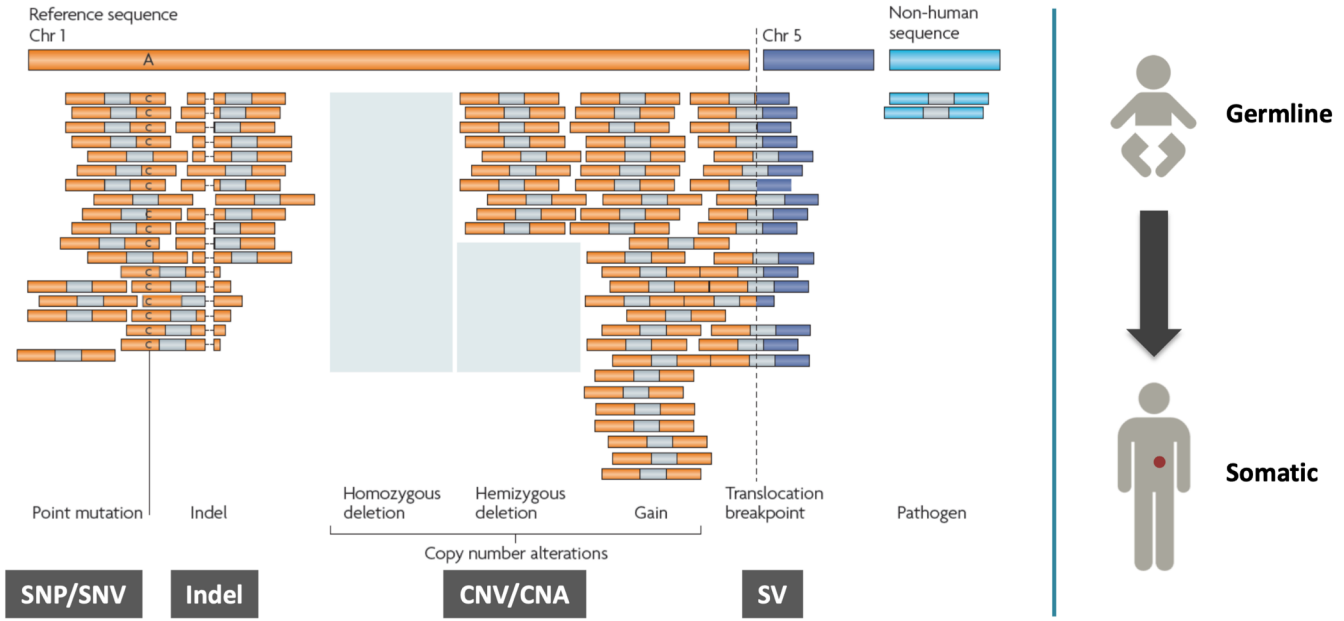


https://www.bcgsc.ca/news/genome-sequencing-helps-prioritize-cancer-treatment-options

Precision cancer medicine

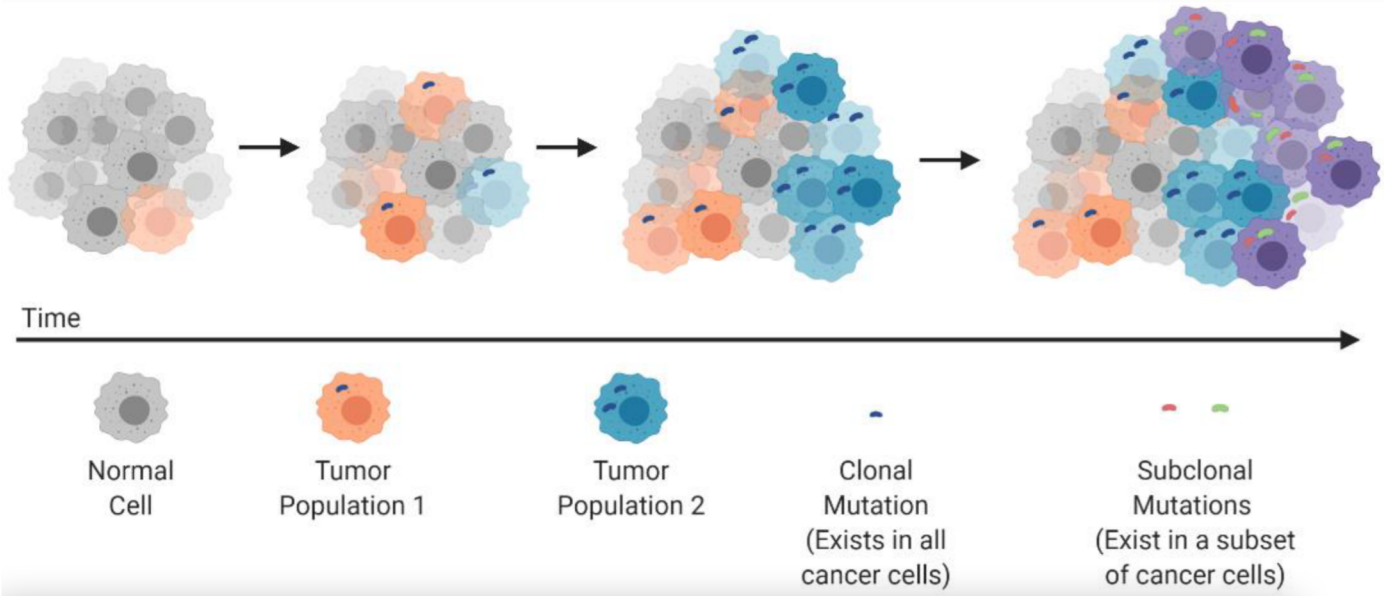


Cancer: multiple types of DNA aberrations



Why is somatic variant calling so challenging?

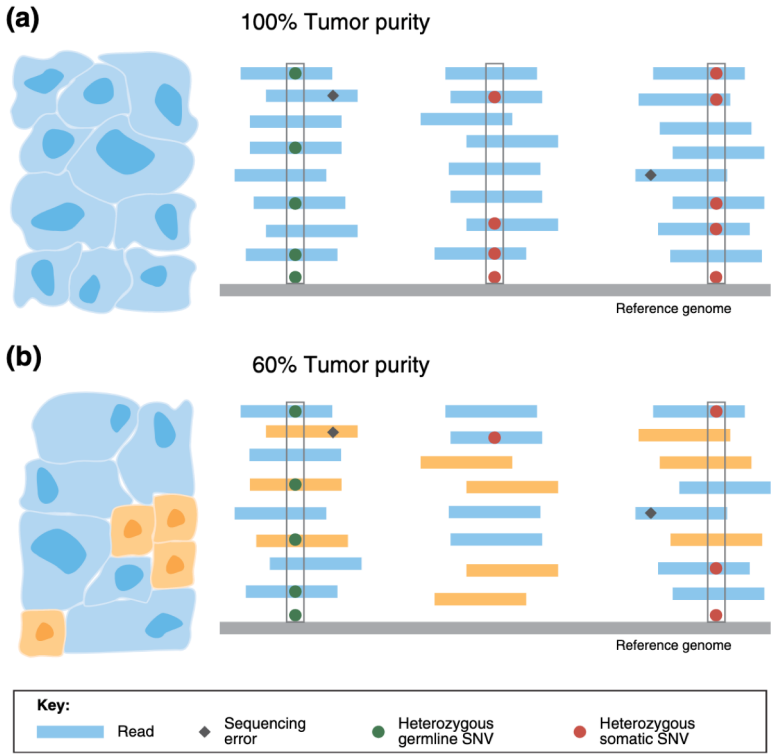
Cancer complexity - tumor purity and heterogenity (I)



El-Sayes et al., Cancers, 2021

- Cancer sequencing provides a snapshot in time and space

Cancer complexity - tumor purity and ploidy (II)



Raphael et al., Genome Med, 2014

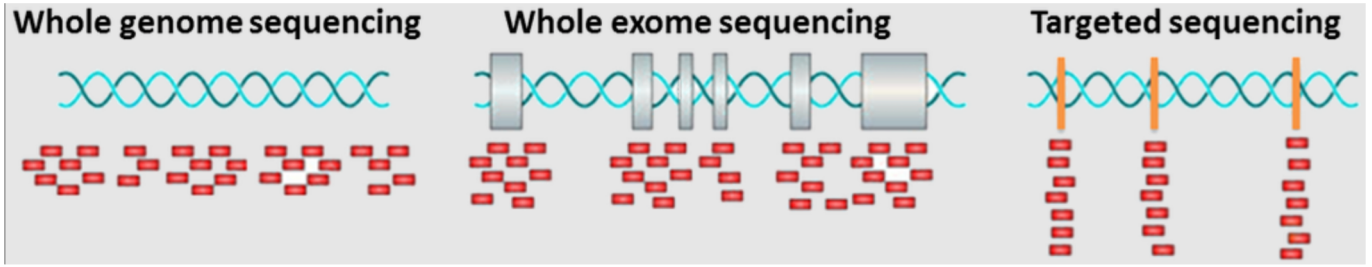
$$Tumor\ purity = \frac{tumor\ cells}{(normal + tumor\ cells)}$$

- Detection capability of low-frequency alleles
- Implications for targeted sequencing design

- Purity is traditionally assessed manually by pathologists, but can also be inferred computationally

- **Aneuploidy**, an imbalanced complement of chromosomes – hallmark of cancer cells

Cancer sequencing: assay design



- Typically 30-40x coverage
- More even coverage than WES
- Covers coding and non-coding/regulatory variation
- All types of variants (reliable detection of SVs)

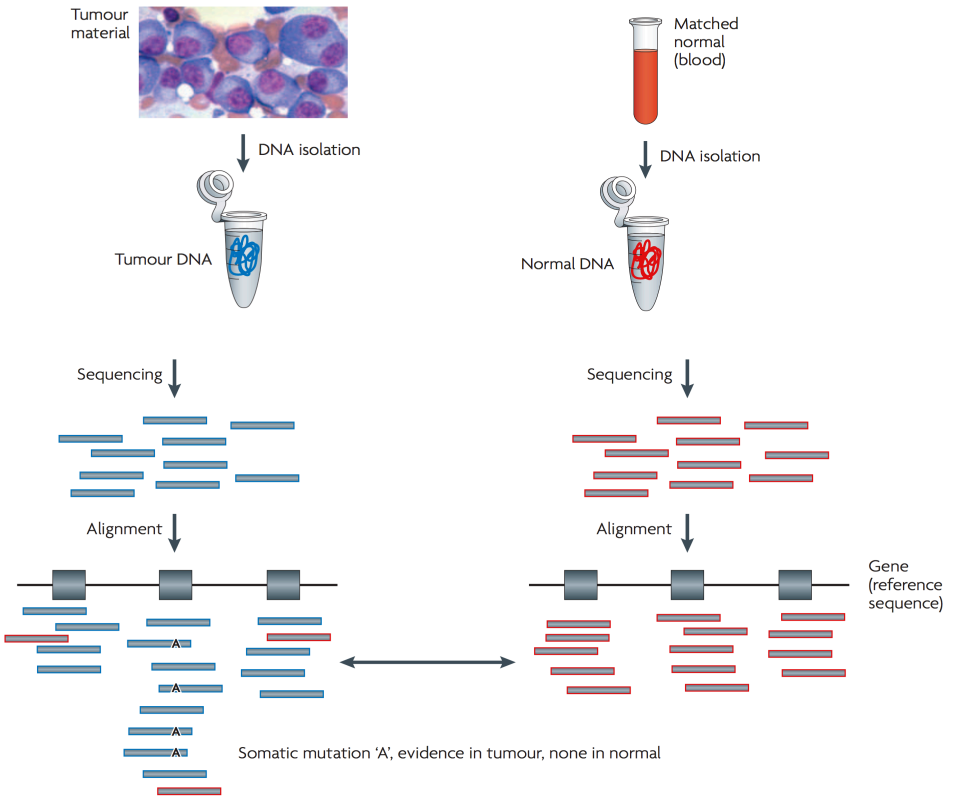
- Typically 80-100x coverage
- Coding regions only
- Cost-effective

- Typically > 300X coverage – captures subclonal variants at low allele frequencies
- Custom target regions – i.e. cancer-relevant/clinically actionable genes
- Most cost-effective

Research ⟵ – – – – – – – – ⟶ *Clinical applications*

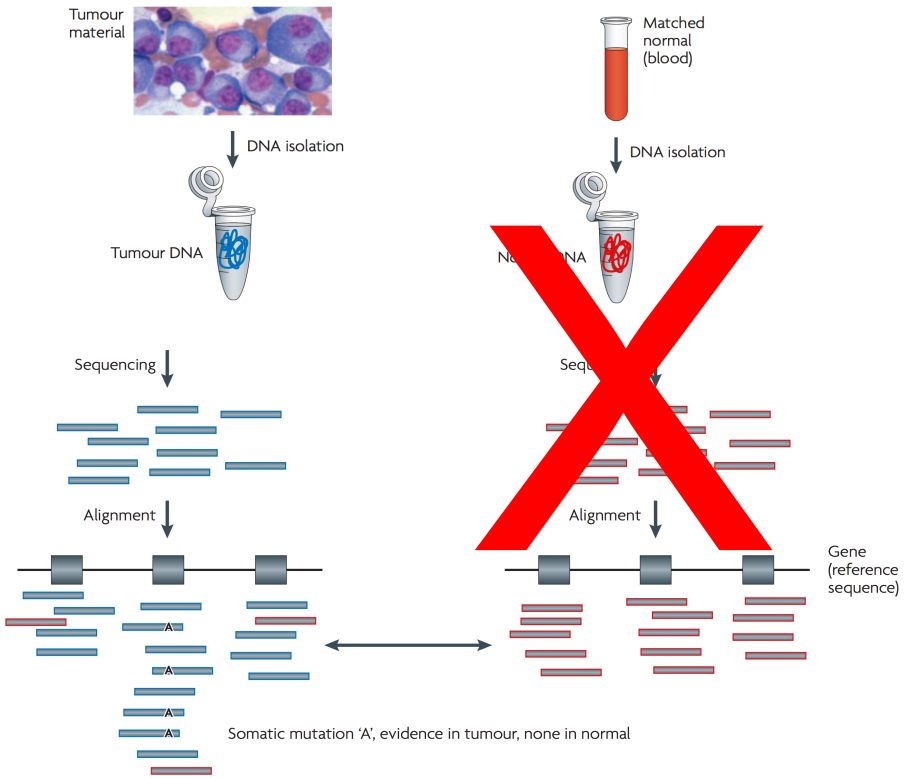
Cancer sequencing: somatic variant calling design

- Two typical sequencing designs for detection of somatic variants
  - Tumor-control (**T + N**): most accurate

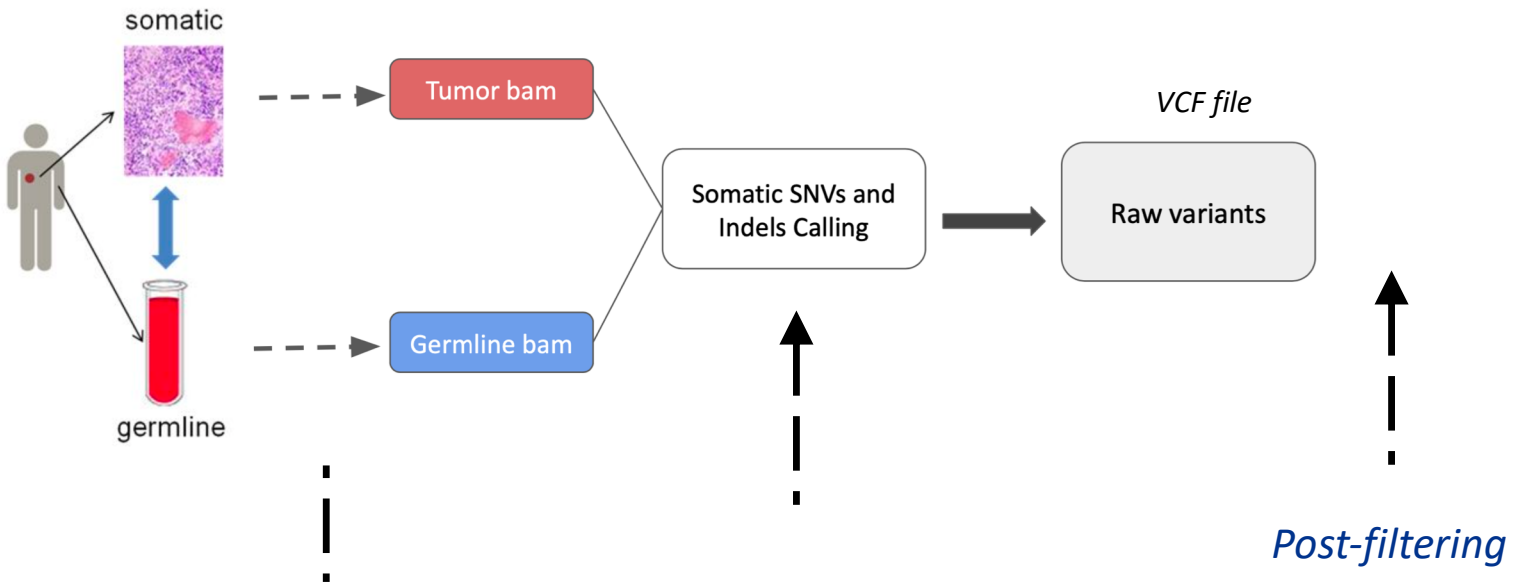


Cancer sequencing: somatic variant calling design

- Two typical sequencing designs for detection of somatic variants
  - Tumor-control (**T + N**): most accurate
  - Tumor-only: most cost-effective

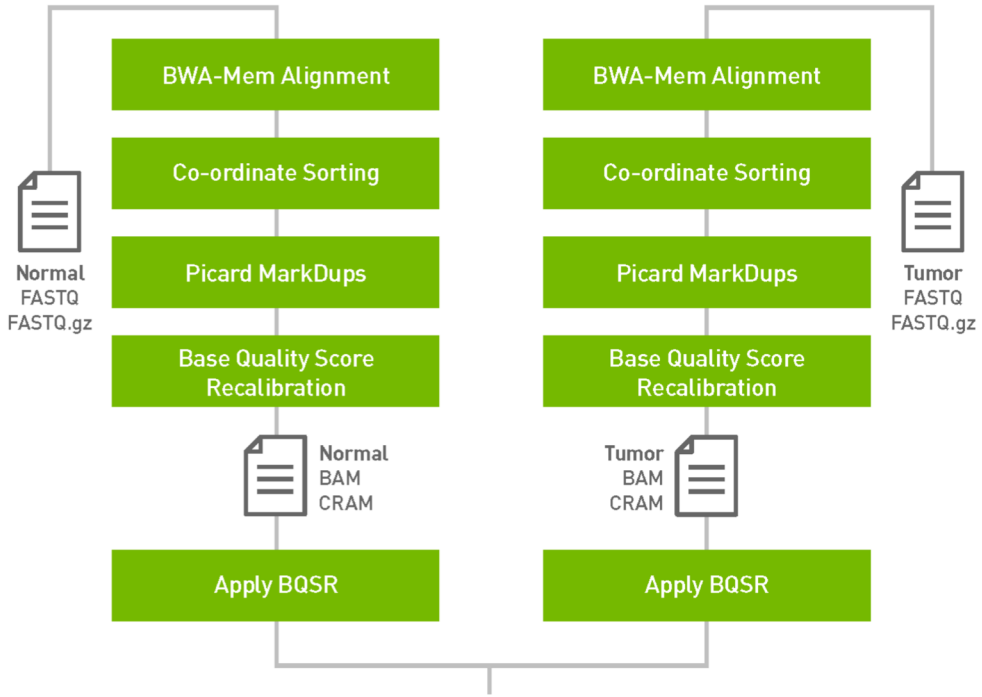


Somatic variant calling



Somatic variant calling: pre-processing

Preprocessing
|
Quality control
|
Variant evaluation
|
Post-filtering



https://docs.nvidia.com/clara/parabricks/v3.0/

Quality control (I)

Preprocessing
|
Quality control
|
Variant evaluation
|
Post-filtering

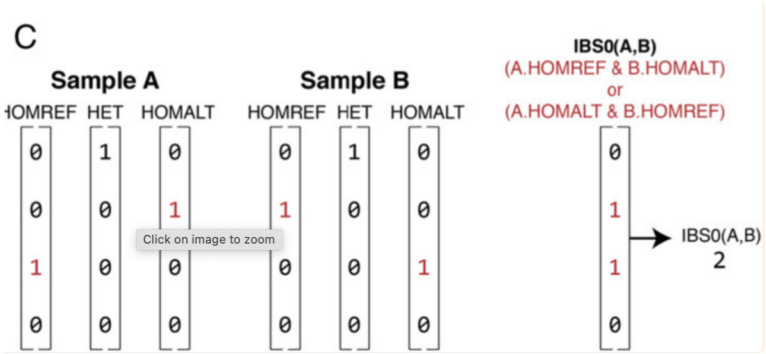
- Tumor samples subject to chemical DNA damage during sample preparation could confound variant identification
  - Oxidation-induced C>A:G>T variants

- Detection?
  - Imbalance between complementary nucleotide substitutions
  - Tools: **GATK**

Quality control (II)

Preprocessing
|
Quality control
|
Variant evaluation
|
Post-filtering

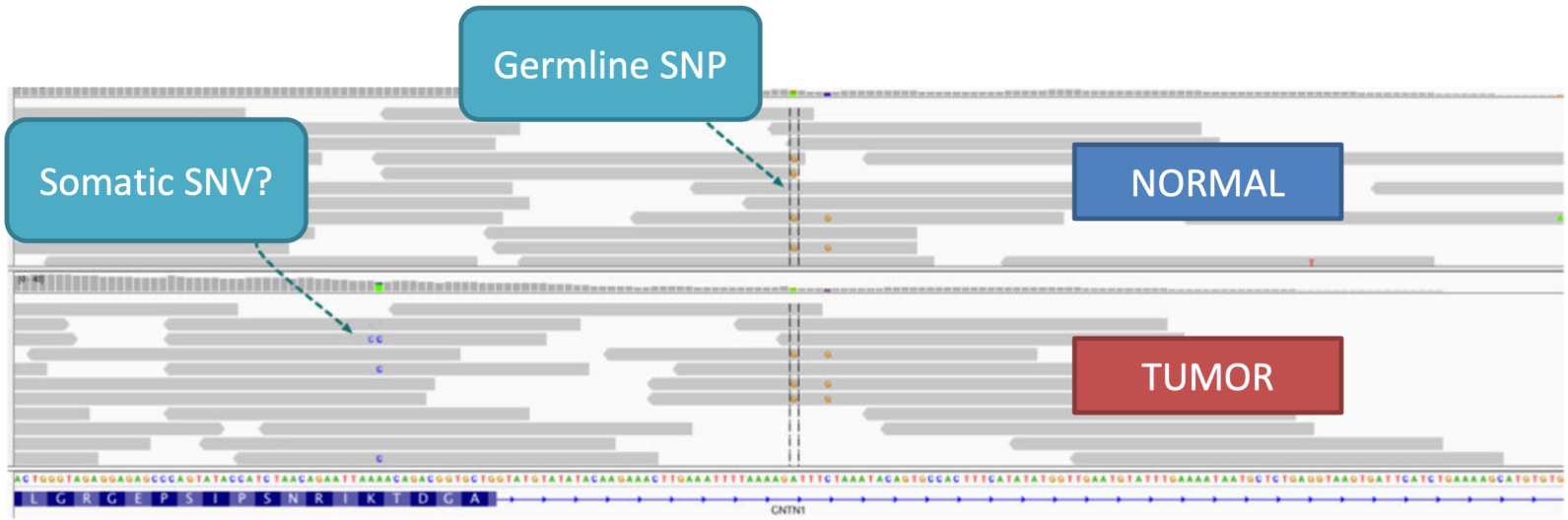
- Cross-sample contamination and sample relatedness
  - Different samples are frequently handled/sequenced together
    - Cross-individual contamination may occur, even small levels of contamination will have an impact on somatic variant detection
  - **T + N:** Check that tumor and normal sample come from the same individual!
  - Tools: **Conpair/Somalier**



Pedersen et al., Genome Med, 2020

Somatic variant calling (T + N)

Preprocessing
|
Quality control
|
Variant evaluation
|
Post-filtering



- Underlying principle for somatic variant calling algorithms using tumor-normal design: **"subtract" the germline background**
  - For a given candidate site, is the difference between tumor and normal significant?

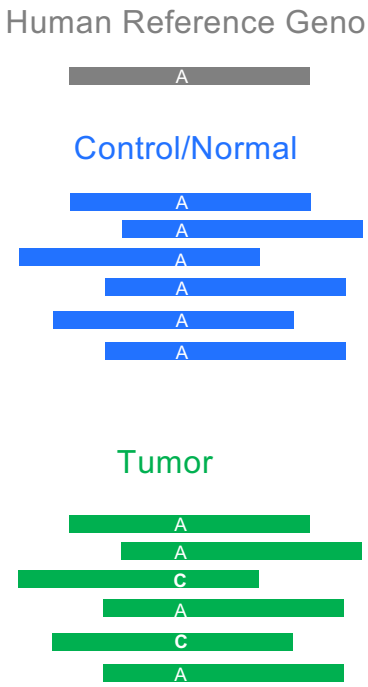
Somatic variant calling (T + N)

Preprocessing
|
Quality control
|
Variant evaluation
|
Post-filtering

- ***First generation***: call somatic candidates through heuristic rules/ad-hoc filters
  - Rule out sequencing artefacts by thresholds (number of supporting reads etc)
  - Does the read count distribution support a variant (and not noise)?
  - Callers: **VarScan, VarDict**

- ***Second generation***: probabilistic modeling of allele frequencies
  - Hypotheses/prior probabilities and likelihood estimation
  - Callers: **MuTect2, Strelka2**
  - ***Post-filtering***: add additional quality control on call set to remove false positives (mapping quality, unbiased strand distribution etc.)

Somatic variant calling (T + N)



Preprocessing
|
Quality control
|
Variant evaluation
|
Post-filtering

MuTect2: Likelihood calculation for different scenarios

Hypothesis 1: Somatic Variant Model

- The tumor sample has a mutation at a site that is absent in the normal sample.

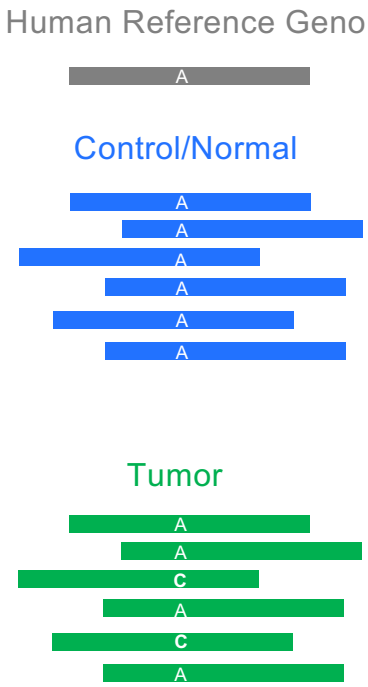
Hypothesis 2: Germline Variant Model

- A variant is present in both the tumor and normal samples (at same frequency), indicating it is inherited rather than acquired.

Hypothesis 3: Error Model

- The observed variant in the tumor sample is due to sequencing error or other artifacts.
- The likelihood of this model depends on sequencing quality metrics like read depth, base quality, and strand bias.

Somatic variant calling (T + N)

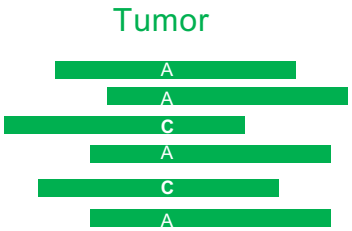


Preprocessing
|
Quality control
|
Variant evaluation
|
Post-filtering

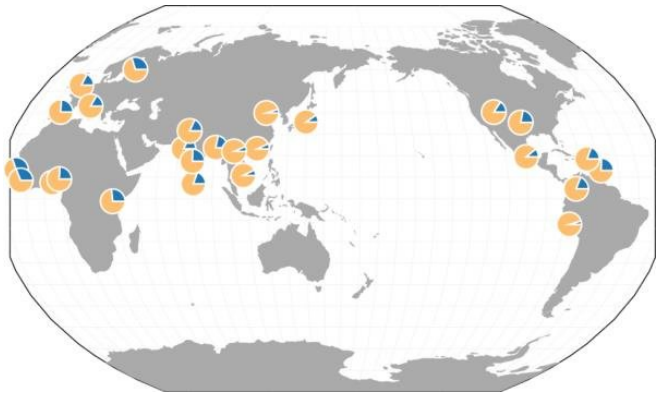
MuTect2: choose best hypothesis through Bayesian inference

- Prior probabilities (somatic mutations, germline variant, sequencing errors)

Allelic fraction versus allele frequency



Variant allelic fraction = 2 / 6



Population allele frequency

- *rs6602666, A>G*
- *Finnish, 0% allele frequency*
- *African 26% allele frequency*

Somatic variant calling (T + N)

- Multiple callers exist - how to choose variant calling algorithm for a particular sequencing project?
  - Check out benchmarking results
    - A few benchmarking datasets are available – providing “gold sets” of somatic mutations
    - **Precision vs. recall**
    - Benchmarking results may be misleading
      - Which calling parameter values should be used?
  - Check whether the algorithm is designed for your assay and technology
    - E.g. has it shown good performance for detection of subclonal variants at low allele frequencies?

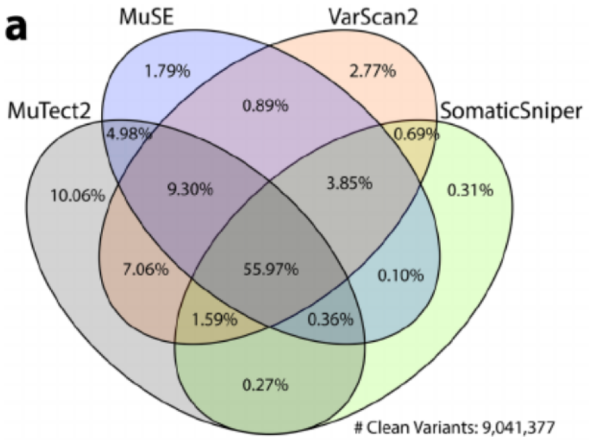

nature COMMUNICATIONS

A comprehensive assessment of somatic mutation detection in cancer using whole-genome sequencing

Tyler S. Alioto, Ivo Buchhalter, Sophia Derdak, Barbara Hutter, Matthew D. Eldridge, Eivind Hovig, Lawrence E. Heisler, Timothy A. Beck, Jared T. Simpson, Laurie Tonon, Anne-Sophie Sertier, Ann-Marie Patch, Natalie Jäger, Philip Ginsbach, Ruben Drews, Nagarajan Paramasivam, Rolf Kabbe, Sasithorn Chotewutmontri, Nicolle Diessl, Christopher Previti, Sabine Schmidt, Benedikt Brors, Lars Feuerbach, Michael Heinold, Susanne Gröbner, Andrey Korshunov, Patrick S. Tarpey, Adam P. Butler, Jonathan Hinton, David Jones, Andrew Menzies, Keiran Raine, Rebecca Shepherd, Lucy Stebbings, Jon W. Teague, Paolo Ribeca, Francesc Castro Giner, Sergi Beltran, Emanuele Raineri, Marc Dabad, Simon C. Heath, Marta Gut, Robert E. Denroche, Nicholas J. Harding, Takafumi N. Yamaguchi, Akihiro Fujimoto, Hidewaki Nakagawa, Víctor Quesada, Rafael Valdés-Mas, Sigve Nakken, Daniel Vodák, Lawrence Bower, Andrew G. Lynch, Charlotte L. Anderson, Nicola Waddell, John V. Pearson, Sean M. Grimmond, Myron Peto, Paul Spellman, Minghui He, Cyriac Kandoth, Semin Lee, John Zhang, Louis Létourneau, Singer Ma, Sahil Seth, David Torrents, Liu Xi, David A. Wheeler, Carlos López-Otín, Elias Campo, Peter J. Campbell, Paul C. Boutros, Xose S. Puente, Daniela S. Gerhard, Stefan M. Pfister, John D. McPherson, Thomas J. Hudson, Matthias Schlesner, Peter Lichter, Roland Eils, David T. W. Jones & Ivo G. Gut - Show fewer authors

Somatic variant calling (T + N)

- How to choose variant calling algorithm for a particular sequencing project?
  - Each caller typically has some strengths and weaknesses
    - a common strategy is to **apply multiple callers and combine the variant sets**
    - "The wisdom of crowds"
    - Union vs. intersection
      - Balance between precision and recall
    - Combining information from VCF files/callers are frequently challenging in practice



Zhang et al., Nat Commun, 2021

Somatic variant calling: VCF

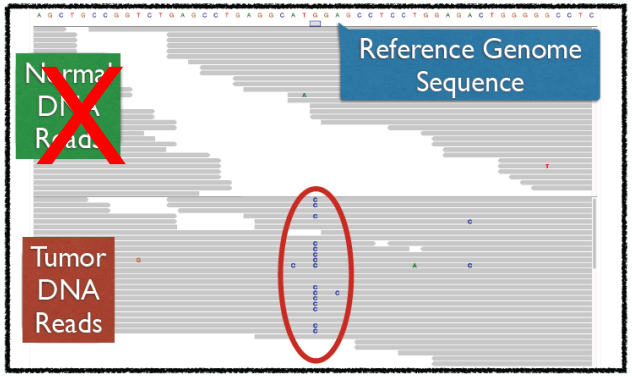
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT CPCT02080287R CPCT02080287T

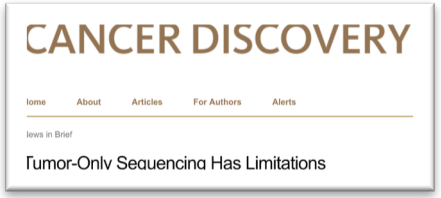
1 854389 . G A 590 **PASS**
IMPACT=LINC02593,ENST00000609207,non_coding_transcript_exon_variant,NONE,false,n.2008C>T,,,NONE,1
GT:**AD:AF:DP**:RABQ:RAD:RC_CNT:RC_IPC:RC_JIT:RC_QUAL:RDP:SB 0/0:**42,0**:**0**:**42**:1528,0:42,0:0,0,0,0,0,42,42:0:0,0,0:0,0,0,0,0,1097,1097:42:0 0/1:**43,20**:**0.317**:**63**:1626,741:46,21:17,3,0,0,0,43,63:0:0,0,0:533,57,0,0,0,1318,1908:67:0.35

Allelic support – tumor sample

Allelic support – normal sample

Somatic variant calling: Tumor-only



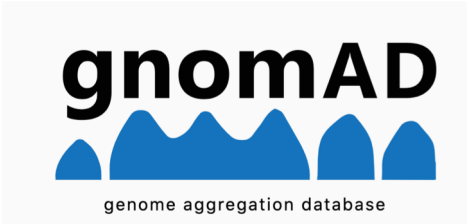


- Cost-effective strategy for identification of somatic variants – much used in the clinic
- Main challenge: robust subtraction/filtering of the germline background
  - Approach: use other sources of germline variation (databases)
  - Each individual carries also private variants (i.e. *singletons*)
  - Ethnic subpopulations are under-represented in germline variant databases

- Targeted sequencing assays/known cancer hotspots – *a priori* assumption that findings are somatic/cancer-relevant

Tumor-only variant filtering: **gnomAD**

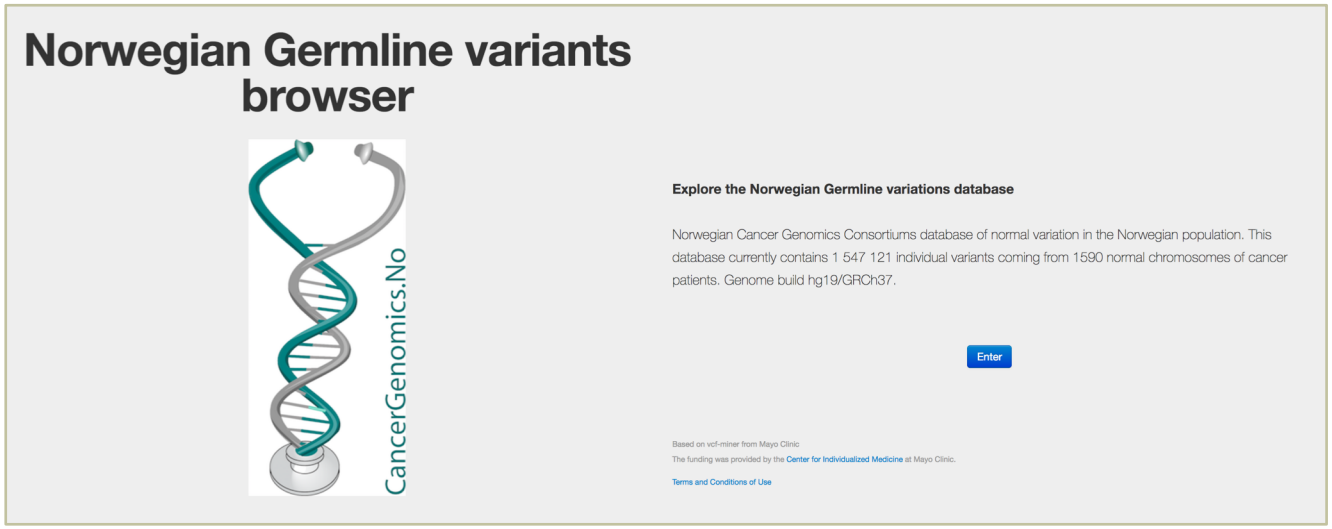
- genome **A**ggregation **D**atabase
- **Harmonizes** germline variant data, both exome and genome sequencing, from a wide variety of large-scale sequencing projects
- **Freely available** to the scientific community
- ~735,000 WES samples
- ~75,000 WGS samples



	ExAC	gnomAD v2	gnomAD v3	gnomAD v4*		
	#	#	#	#	%	Fold increase from v2
Admixed American	5,789	17,720	7,647	30,019	3.72%	1.7x
African	5,203	12,487	20,744	37,545	4.65%	3x
Ashkenazi Jewish	-	5,185	1,736	14,804	1.83%	2.9x
East Asian	4,327	9,977	2,604	22,448	2.78%	2.3x
European^	36,667	77,165	39,345	622,057	77.07%	8.1x
Middle Eastern	-	-	158	3,031	0.38%	New
Remaining Individuals^	454	3,614	1,503	31,172	3.93%	8.8x
South Asian	8,256	15,308	2,419	45,546	5.64%	3x
Total	**60,706**	**141,456**	**76,156**	**-**	**807,162**	**-**

*v4 includes all v3 samples
^ Due to small sample sizes Finnish was included in European and Amish was included in Remaining Individuals

Tumor-only variant filtering: **norgene**



norgene.no

Tumor-only variant filtering: **panel-of-normals**

- What is a «panel-of-normals (PON)»?
  - Variant calls made from a set of unrelated “normal” samples
- Purpose of PON?
  - Eliminate common/recurring technical artifacts
    - should use normals made using the same data generation techniques (e.g. same capture kit for exomes, same sequencing platform etc.)
  - Secondary purpose: also eliminates germline variants not called in the matched normal (or approximates the normal if none is available)

Somatic variant calling: summary

- The complexity of tumors pose challenges for variant identification – intratumor heterogeneity, tumor purity, ploidy
- WGS – WES – Targeted sequencing (research → clinic)
- Two fundamental sequencing designs: Tumor-control and tumor-only
- Multiple calling algorithms exist – each with strengths and weaknesses - a common strategy is to combine output from several callers
- Benchmarking results exist – can they be generalized?
- Understand the nature of your data/tumor and the priorities of the variant identification procedure when choosing a calling strategy

Variants have been found – now what?

ACTG**C**CTACGTCTCACCGTCGACTTCAAATCG**C**TTAACCCGTACTCCCATGCTACTGC
ATCTCGGGTTAACTCGACGTTTT**T**CATGCATGTGTGCACCCCAATATATATGCA**A**CTT
TTGTGCACCTCTGTCACGCGCGAGTTGGCACTGTCGCCCCTGTGTGCATGTGCACTGT
CTC**T**CGCTGCACTGCCTACGTCTCACCGTCGACTTCAAATCG**C**TTAACCCGTACTCCC
ATGCTACTGCATCTCGGGTTAACTCGACGTTTT**G**CATGCATGTGTGCACCCCAATATA
TATGCA**A**CTTTTGTGCACCTCTGTCACGCGCGAGTTGGCACTGTCGCCCCTGTGTGCA
TGTGCACTGTCTC**T**CGAGTTTT**G**CATGCATGTGTGCACTGTGCACCTCTGTTACGTCT

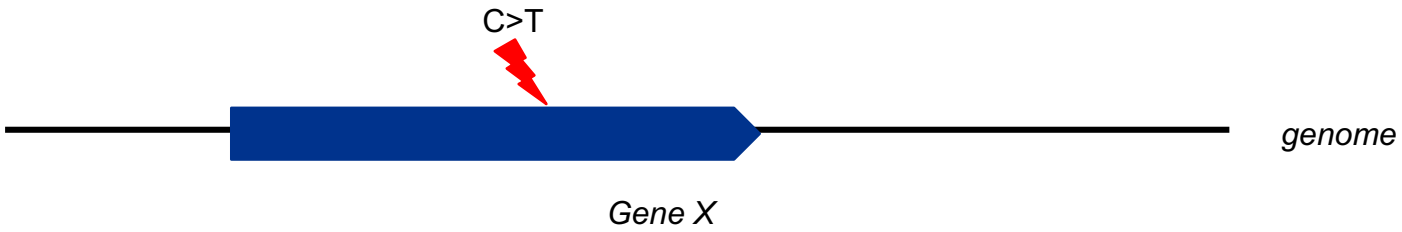
QUESTIONS/BREAK

Learning outcomes – part II

- Important cancer variant annotation concepts
  - Tumor suppressor genes versus proto-oncogenes
  - Gain-of-function versus loss-of-function variation
- Guidelines/operating procedures for variant classification and prioritization in cancer
  - Oncogenicity
  - Clinical actionability
- Mutation landscapes - «complex» biomarkers

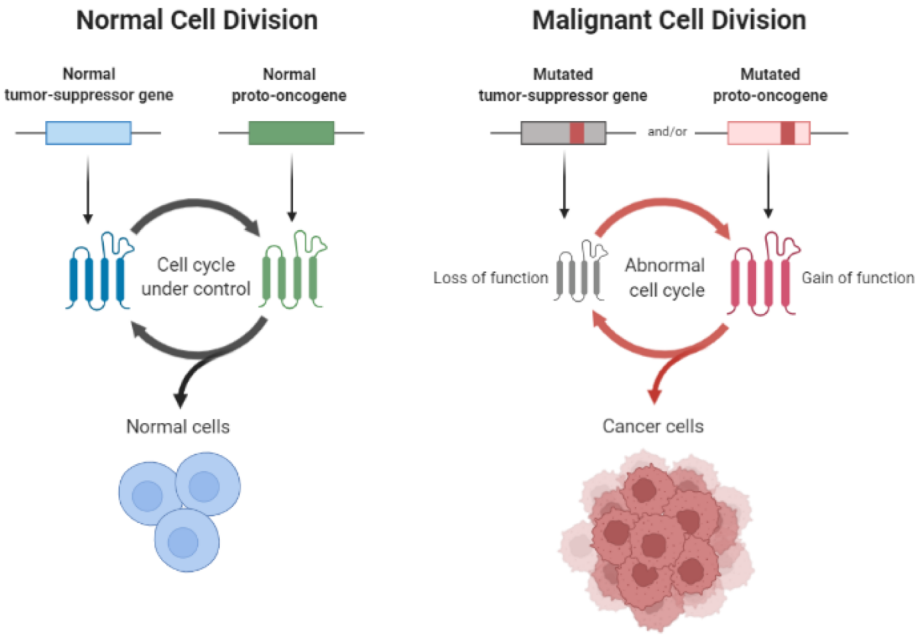
- *Perspective – precision medicine/single sample analysis*

Individual variant interpretation – key questions



1. Which gene is mutated?
   - Is the gene cancer-relevant?
2. What is the predicted consequence for the encoded protein?
   - Loss-of-function?
   - Gain-of-function?
3. Is it actionable?

Which genes are mutated (II)?



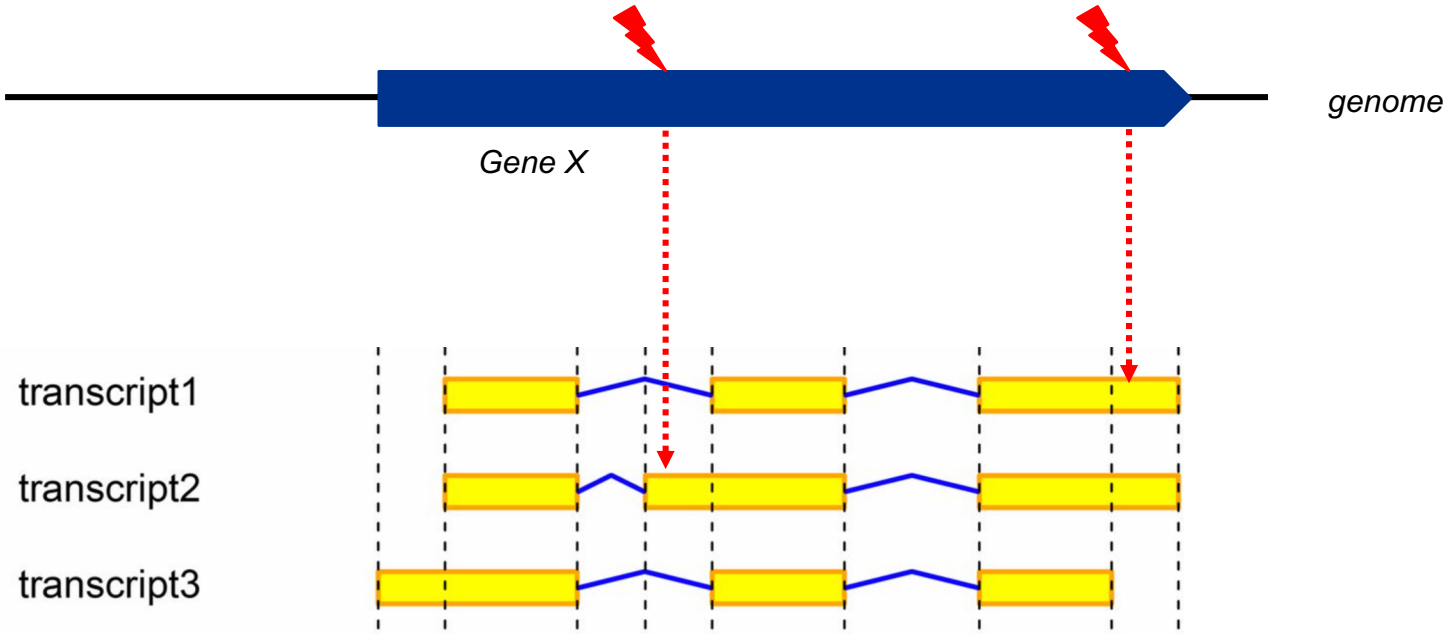
- N = 360 proto-oncogenes
- N = 372 tumor suppressor genes

Cancer Gene Census

https://cancer.sanger.ac.uk/census

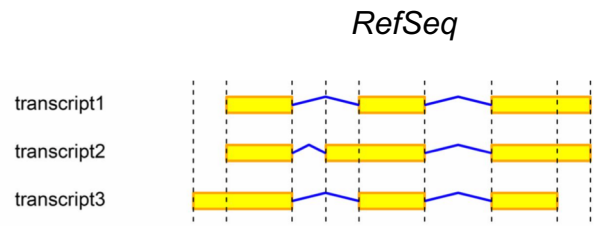
Variant consequence interpretation - general

- Important: a gene typically consists of multiple transcript isoforms

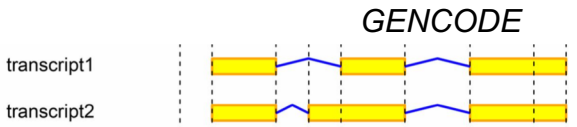


Variant consequence interpretation – general (II)

- Several transcript/gene model databases
  - RefSeq
  - Ensembl
  - GENCODE
    - MANE

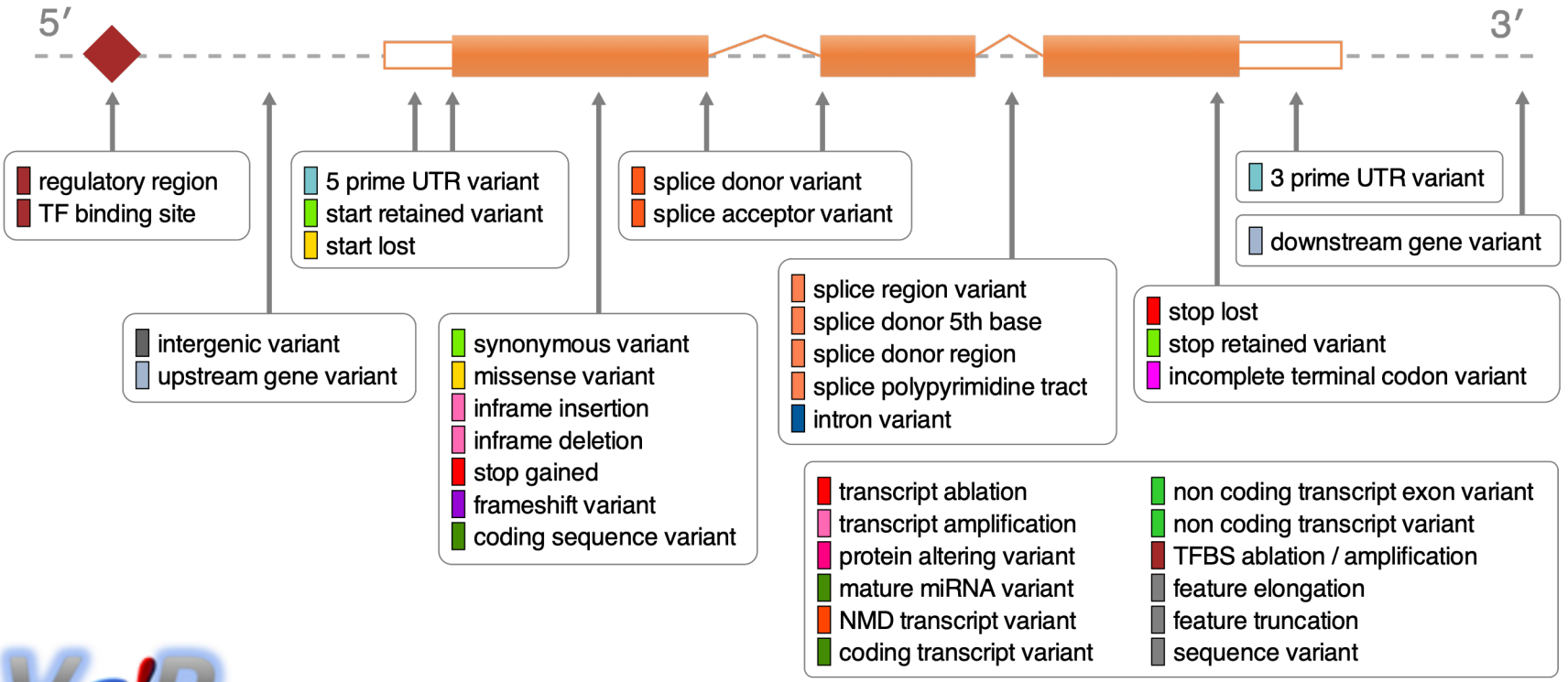


- Choice of transcript database impacts variant consequence/ annotation



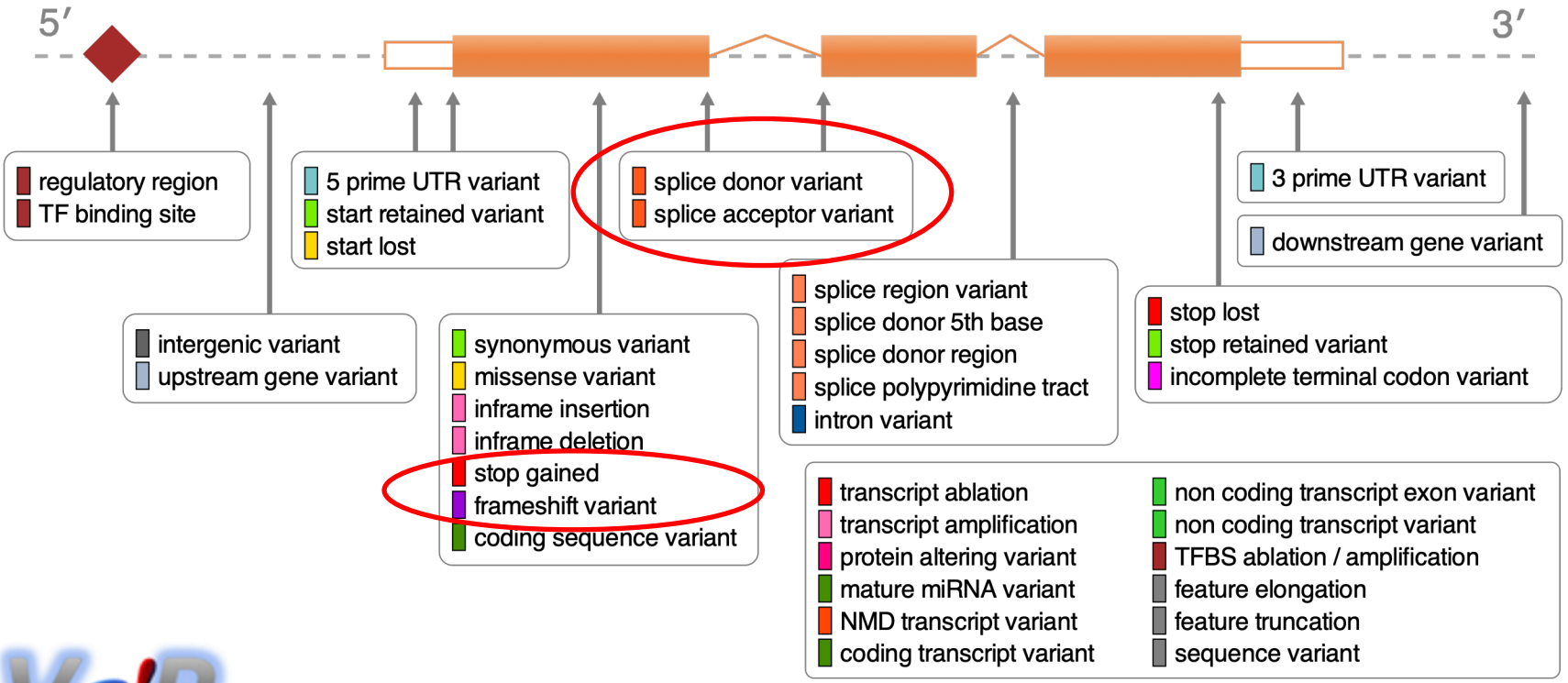
- Frequent strategy: Report variant consequence in most commonly expressed isoform (i.e. ***principal*** isoform)

Variant consequence interpretation – general (III)



Variant Effect Predictor

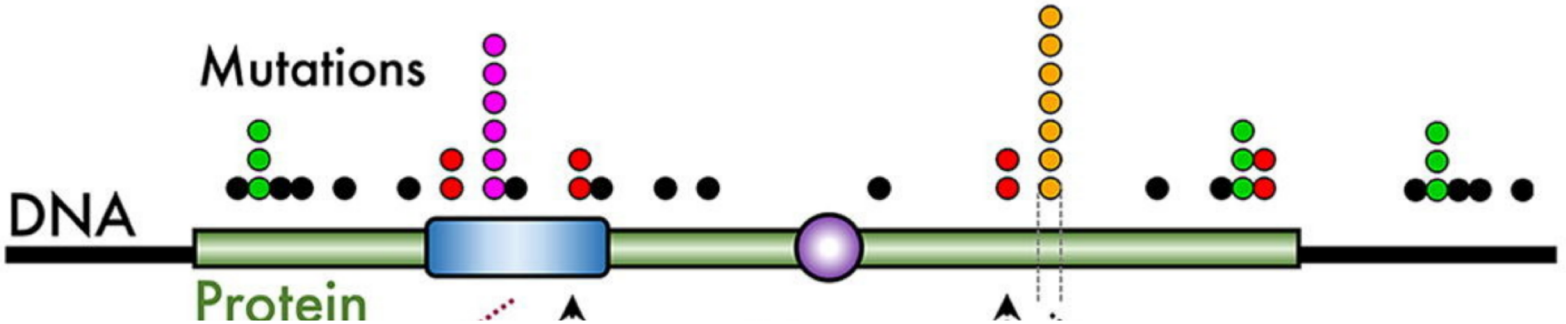
Loss-of-function variants



Variant Effect Predictor

Gain-of-function variants

- Much harder to predict than loss-of-function variants
- Large-scale sequencing efforts have found clusters of missense mutations in oncogenes



Cancer Hotspots

cancerhotspots.org

Variant oncogenicity classification

- Which tumor variants are likely to be oncogenic?
  - Loss-of-function in tumor suppressor genes, gain-of-function of oncogenes
  - Guidelines/standard operating procedures for classification published

SPECIAL ARTICLE | VOLUME 24, ISSUE 5, P986-998, MAY 2022 Download Full Issue

Standards for the classification of pathogenicity of somatic variants in cancer (oncogenicity): Joint recommendations of Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC), and Variant Interpretation for Cancer Consortium (VICC)

Peter Horak • Malachi Griffith • Arpad M. Danos • ... Obi L. Griffith • Debyani Chakravarty • Dmitriy Sonkin • Show all authors

Open Archive • Published: January 28, 2022 • DOI: https://doi.org/10.1016/j.gim.2022.01.001 •

Check for updates

Variant oncogenicity classification

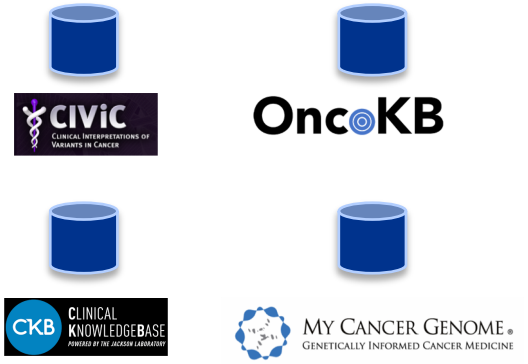
	Benign			Oncogenic			
Evidence Strength	Very Strong	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
POINTS	-8	-4	-1	+1	+2	+4	+8
Population Data	MAF is >5%	MAF is >1%		Absent in population databases			
Functional Data		Well-established functional studies show no oncogenic effects				Well-established functional studies supportive of an oncogenic effect	
Predictive Data			Silent mutation (no predicted impact on splicing)		Missense change at an amino acid residue where a different missense change determined to be oncogenic has been documented	Same amino acid change as a previously established oncogenic mutation	Null variant in tumor suppressor
Cancer Hotspots				Cancer hotspots with low frequency of recurrence	Cancer hotspot with moderate frequency of recurrence	Cancer hotspot with high frequency of recurrence	
Computational Evidence			All utilized lines of computational evidence suggest no impact of a variant	All utilized lines of computational evidence support oncogenicity			

Which variants are actionable? (I)

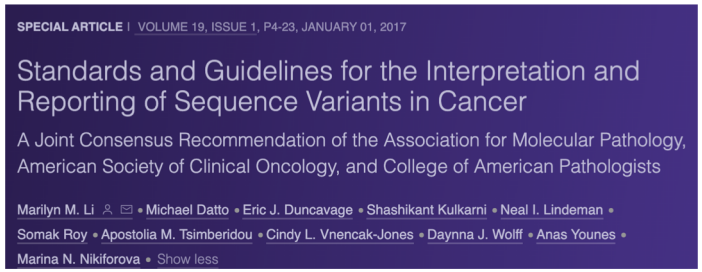
- Specific genetic aberrations indicate clinical actionability
  - **Drug sensitivity**
  - Prognosis / Diagnosis
  - Drug resistance

- Multiple initiatives curate clinical variant associations in cancer
  - **Variant X** in **phenotype Y** indicates sensitivity to **drug Z**
  - **Challenge:** harmonization of knowledge databases
    - VICC (Variant Interpretation for Cancer Consortium)

- *HER2 amplification – Trastuzumab – Breast Cancer*
- *BRAF V600E – Vemurafenib – Melanoma*
- *BRAF V600E – Trametinib + Dabrafenib – NSCLC*
- *IDH1/2 mutations – Ivosidenib – AML*
- ..
- ..



Which variants are actionable? (II)



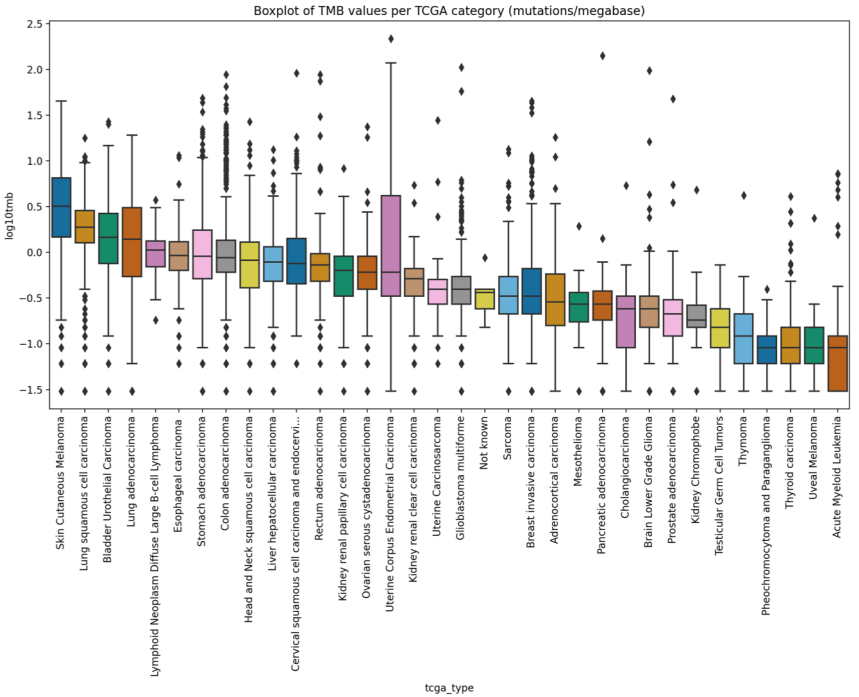
- How do we prioritize variants according to actionability?
  - Classification guidelines - tiers
  - Key: **Strength of evidence**
  - Tumor type (**on-label** vs. **off-label**)

- **TIER 1** – strong evidence for clinical impact, same tumor type as query
- **TIER 2** – strong evidence for clinical impact in other tumor type or weak evidence for clinical impact in query tumor type
- **TIER 3** – uncertain clinical significance; coding variants in tumor suppressor genes/proto-oncogenes (mutation hotspots etc)
- **TIER 4** – other coding variants

Mutation landscapes – complex biomarkers

- Properties of tumor samples not linked to individual variants but rather the global pattern or landscape of mutations found

1. Tumor mutational burden
2. Mutational signatures
3. Microsatellite instability
   - Important in selected tumor types



Mutational burden - how many tumor mutations are found?

- **Tumor mutational burden (TMB)** - number of somatic mutations per megabase of interrogated genomic sequence
- A key driver in the generation of immunogenic neopeptides – influences **response to immune checkpoint inhibitors (ICIs)**
- Clinically significant TMB levels - debatable
- Very simple biomarker – still a main molecular indicator for allocation of patients to immunotherapy

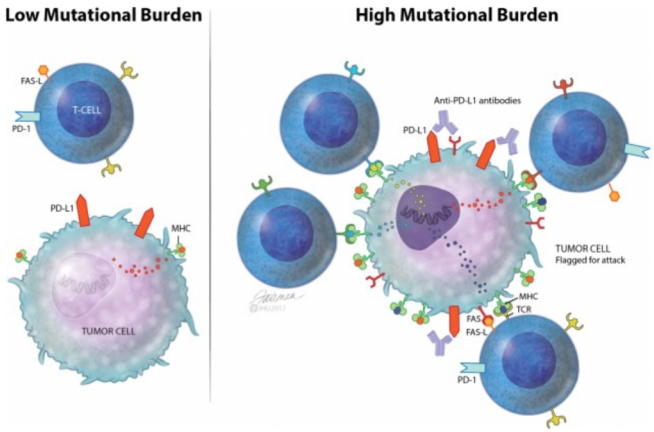


Illustration from Sharabi et al., The Oncologist (2017)

What types of mutations are found?

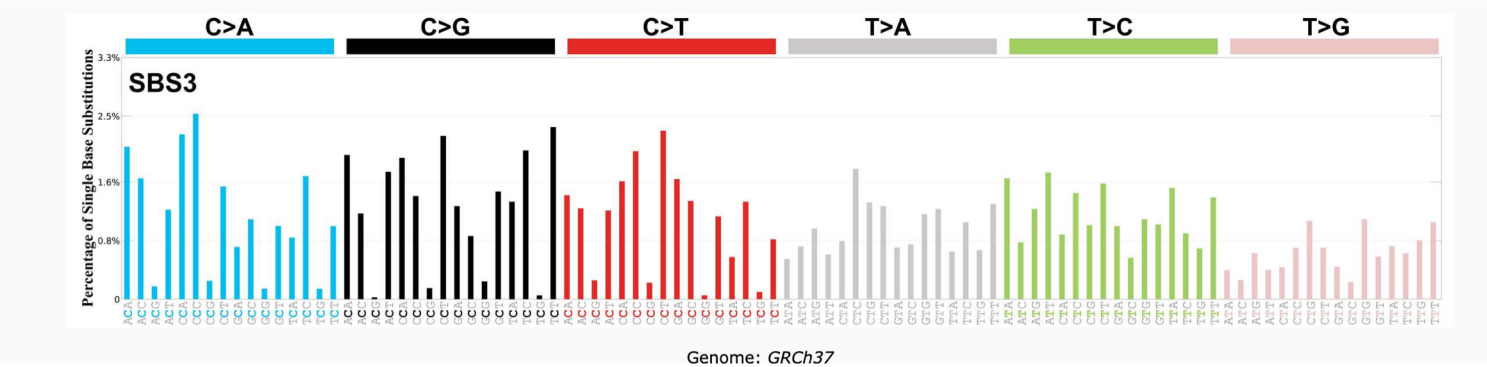
- **Mutational signatures:** characteristic mutation patterns (types and sequence context) that arise from a specific mutational process

- **Premise**: mutational processes are context-dependent (occur non-randomly in DNA)

- **Footprint:** The global set of mutations harvested from NGS **reveals a «historical footprint»** of the mutational processes that have shaped a given tumor
  - Environmental mutagens
  - Endogenous mutation processes (e.g. DNA repair defects)
  - Treatment effects
  - **Approximatly 60 established mutational signatures**

Mutational signatures (I)

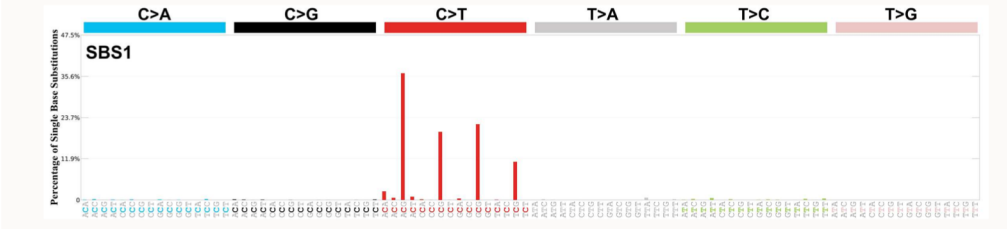
- Mutational signatures are most commonly presented through the **96-channel** approach (single base substitutions, SBS)
  - Mutation type + flanking bases



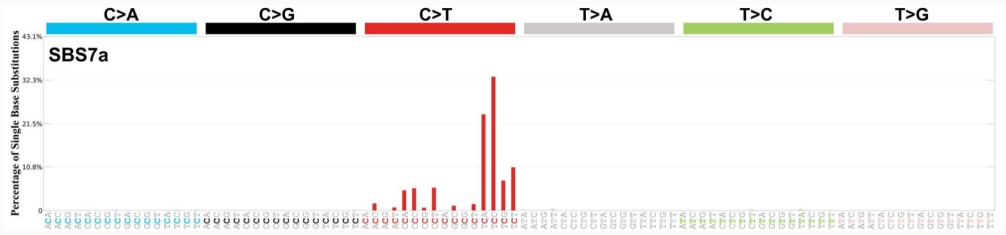
  - A single signature (attributed to a given process) is thus characterized as the **relative frequency of 96 different channels**

Mutational signatures (II)

- Aging
  - spontaneous or enzymatic deamination of 5-methylcytosine to thymine (clock-like signature)

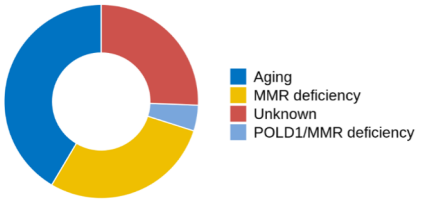


- Exposure to UV light
  - cyclobutane pyrimidine dimers or 6-4 photoproducts



Mutational signatures (III)

- Tools can «deconstruct» the profile of somatic mutations in a tumor towards contribution of known signatures
- Signatures are emerging as an important biomarker for drug response
- Often considered in combination with other markers
- Challenge: **confidence**

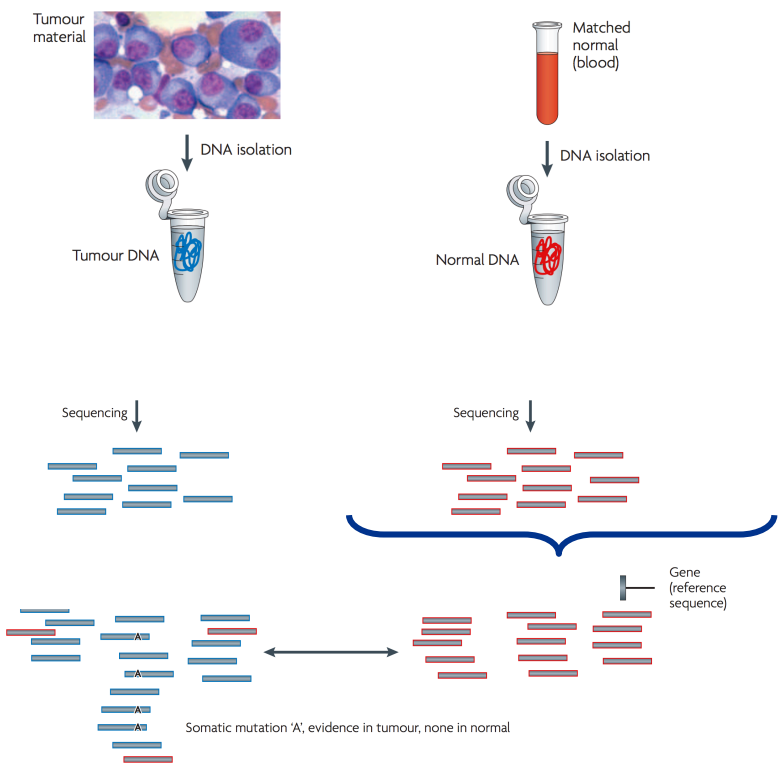


		CS-3	CS-8	Homologous Recombination Repair Deficiency	PARP inhibition[32-34], Platinum-based chemotherapy[35-37]
CS-6	CS-15	CS-20	CS-26	Mismatch Repair Deficiency	PD1-immunotherapy[48-49,52]
		CS-5	CS-8	Nucleotide Excision Repair Deficiency	Cisplatin[63-65]
		CS-18	CS-30	Base excision Repair Deficiency	
			CS-10	Deficient DNA polymerase proofreading activity	PD1-immunotherapy[48-49,52]

Adopted and modified from Van Hoeck et al., BMC Cancer, 2019

Cancer patients - germline background (I)

- Genetic causes of cancer include both somatic mutations and inherited germline variants.
- Approximately 10-15% of all cancers have a hereditary/germline component
- Pathogenic variants in cancer predisposition genes – conferring increased risk of tumor development



Germline variant calling

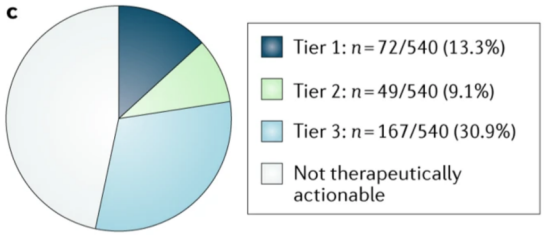
Cancer patients - germline background (II)

- Why important?
  - Implement surveillance and risk-reducing interventions
  - May impact type of surgery (radical /conservative)
  - Targeted therapy implications
    - BRCA (PARP)

nature REVIEWS CLINICAL ONCOLOGY

Review Article | Published: 19 February 2019

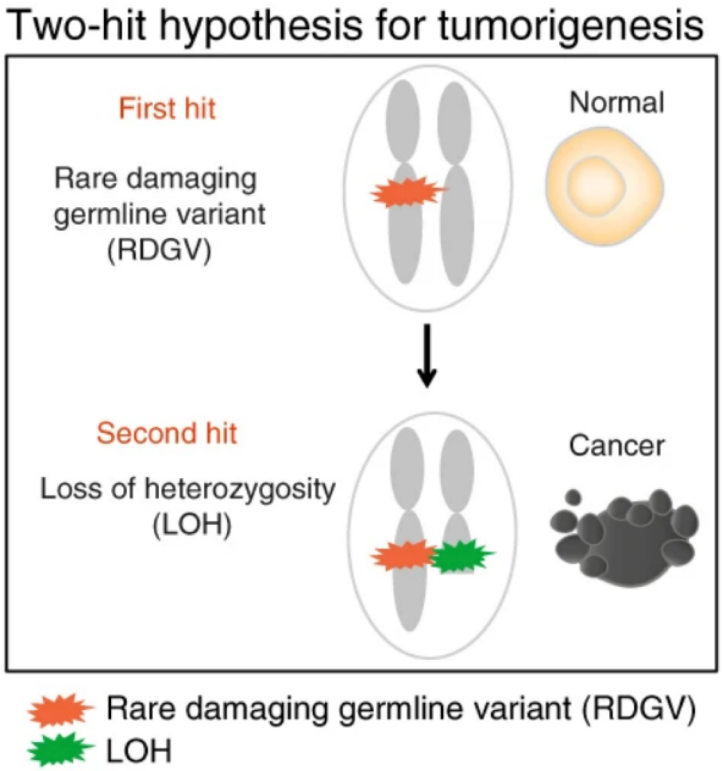
Therapeutic implications of germline genetic findings in cancer



Clinical actionability - TCGA

Cancer patients - germline background (III)

- Knudsen's two-hit hypothesis
  - First formulated in 1971
  - Many tumor suppressor genes require both alleles to be inactivated
  - First mutation inherited, second mutation acquired

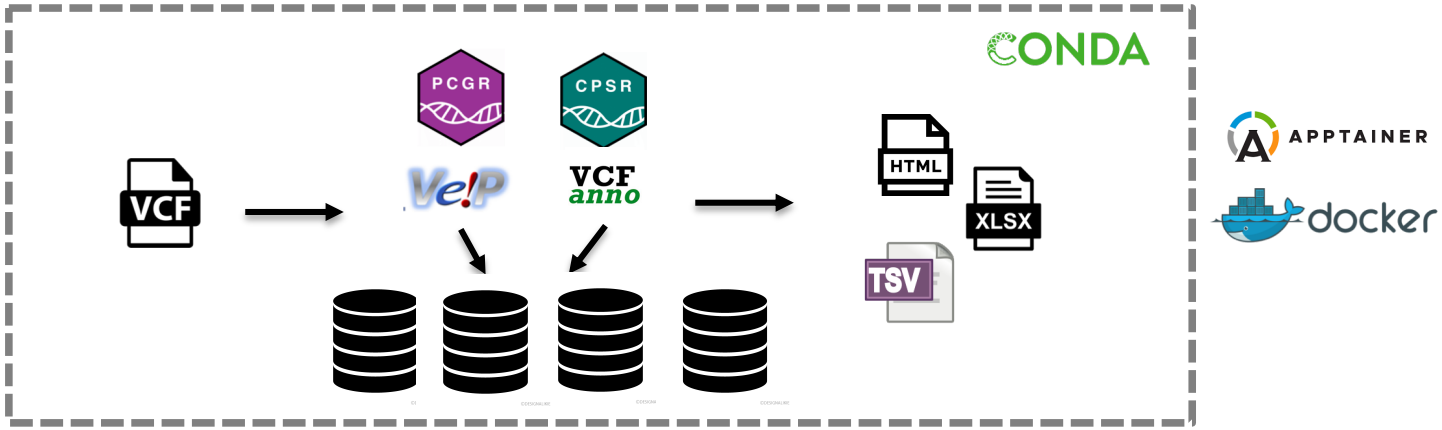


Putting it all together

- **Variant interpretation tools for molecular-based cancer treatment**
  - Personal Cancer Genome Reporter (PCGR): Translation of tumor omics data to measures for clinical decision making
    - Cancer Predisposition Sequencing Reporter (CPSR): Assessment of cancer-predisposing germline variants
  - N-of-one interpretation challenge
    - What's characteristic of tumor X/patient X? Given the current knowledge/reference data
  - Backbone for variant analysis in national precision medicine initatives

PCGR/CPSR

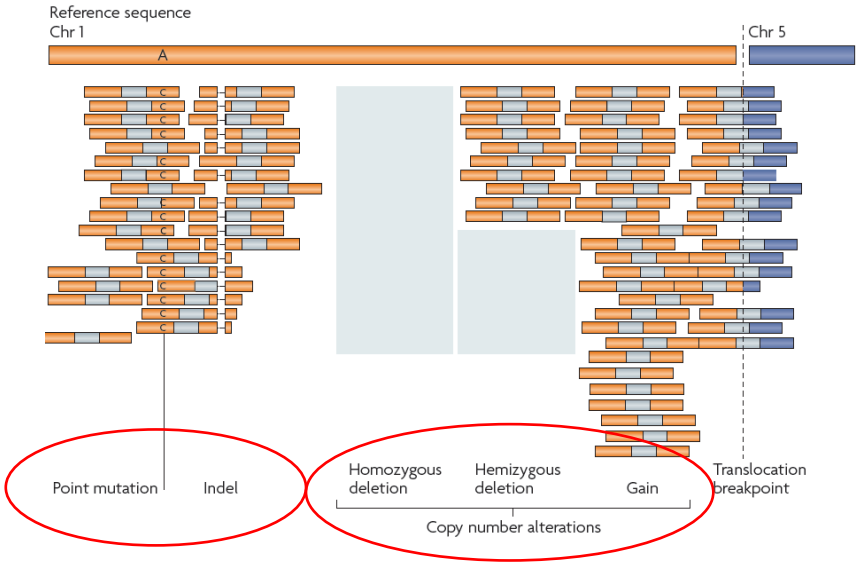
- Overall aim: **harvest precision therapy measures** from high-throughput sequencing data of cancer patients (tumor + germline)
- Focus: variant prioritization/classification
- **Stand-alone reporting engines** – **free**/**open-source** - publicly available reference data
- Reports on a **per-case** basis



PCGR - input data

- Key inputs:

1) Somatic DNA aberrations



Meyerson et al. Nat Rev Genet 2010

2) Tumor type

Breast
Colon/Rectum
Lung
Pancreas
Prostate
…

3) Expression

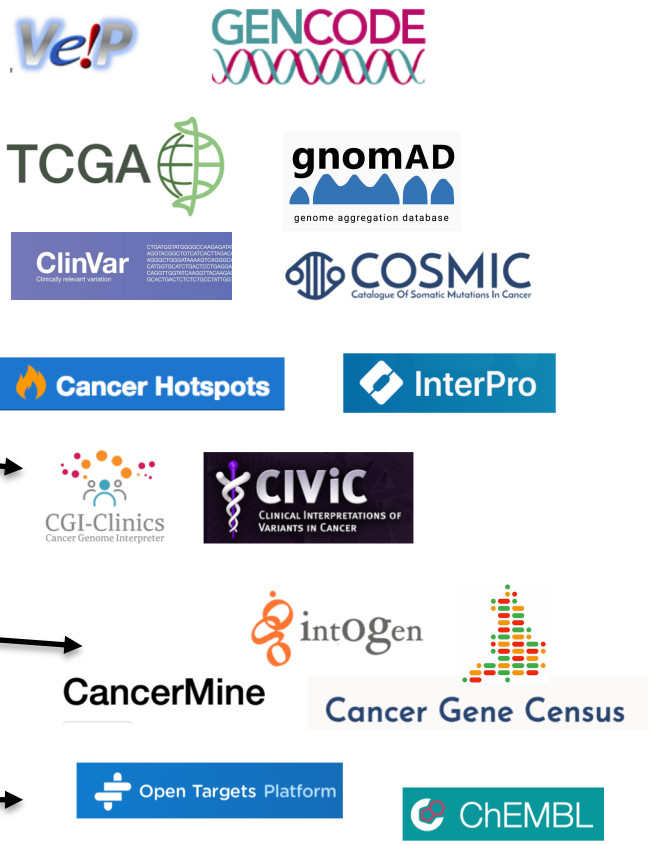
4) Germline findings

5) RNA fusion events
(in progress)

PCGR/CPSR requires prior knowledge

- What type of variant?
  - Which variant consequence?
  - Seen previously? Germline/somatic?

    At what frequency? In which tumor types?
  - Loss-of-function?
  - Gain-of-function/hotspot? In functional domain?
  - Clinically actionable? Drug sensitivity/resistance marker?
- What type of gene?
  - Cancer-relevant? Cancer-predisposing? Known tumor-suppressive or proto-oncogenic role? Part of oncogenic signaling pathways?
  - Molecular drug target? Approved/Late phase/early phase?



PCGR - key functionality

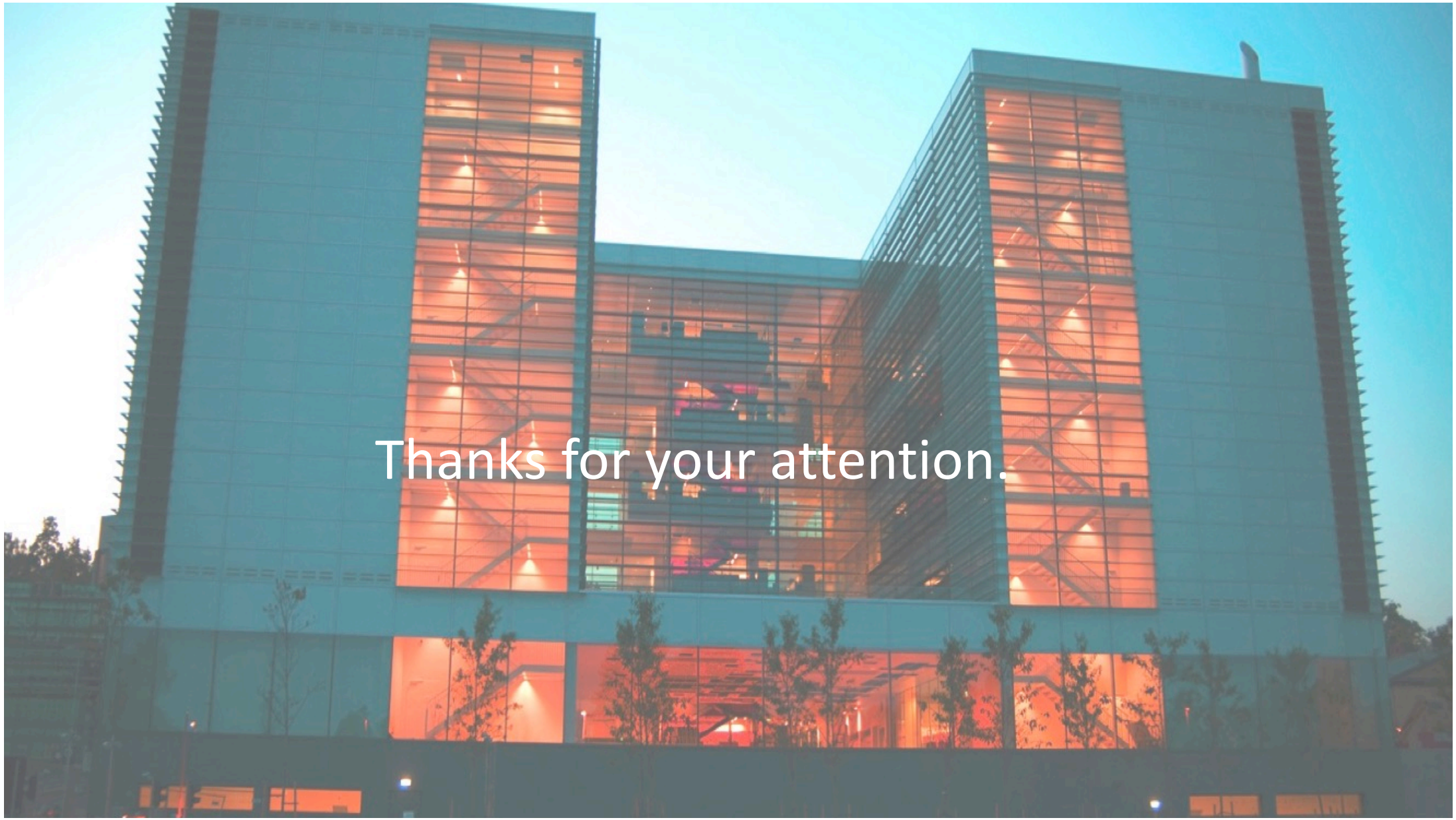
- **A prioritization of variants (SNVs/InDels/CNAs) with respect to functional and clinical impact**
  - Therapeutic actionability/prognosis/diagnosis
  - Oncogenic potential
- Other measures that can guide/inform upon precision treatment
  - **Mutational signatures** – what type of mutational processes have shaped the tumor
  - **MSI classification** – predicted from mutational profile
  - **Tumor mutational burden** – proxy for response to immunotherapy
  - **Germline findings**
  - **RNA expression outliers / RNA similarity analysis**

- Configurable, interactive, and transparent output
- Support for tumor-only and tumor-control input assays
- WES/WGS/targeted assays (TSO500)

PCGR DEMO REPORT

Variant interpretation in cancer: summary

- Comprehensive DNA variant interpretation is critical for implementation of precision cancer medicine
- Types of mutations, number of mutations, mutation locations – all may have therapeutic implications
- Variant consequences are transcript-specific
- A large number of resources have been erected to facilitate clinical interpretation of cancer genomes
- Variant prioritization: tier structure
- Interpretation of the germline background of cancer patients adds an important dimension for clinical translation


Thanks for your attention.